In the Specification:

Please amend the specification as shown:

Please delete the paragraph on page 11, lines 20-26 and replace it with the following paragraph:

Figure 1: is an illustration of the nucleotide **(SEQ ID NO: 34)** and amino acid **(SEQ ID NO: 35)** sequences of human NAALAD-ase L. The nucleotide and predicted single letter code amino acid sequence are shown. the putative membrane spanning domain, deduced from hydrophilicity plots, is marked by a line. Potential N-glycosylation sites are shaded.

Please delete the paragraph on page 11, lines 28-34 and replace it with the following paragraph:

Figure 2: is an alignment of the predicted protein sequences for human **(SEQ ID NO: 35)** and rat **(SEQ ID NO: 36)** NAALAD-ase L. The amino acid sequences were aligned using the ClustalW alignment program (EMBL, Heidelberg Germany). Amino acid residues identical in both proteins are highlighted in black. Amino acid residues are numbered in the right hand margin.

Please delete the paragraph on page 11, line 36 to page 12, line 9 and replace it with the following paragraph:

Figure 3: is an illustration of alternative splicing of NAALAD-ase L. Amino acid sequence for NAALAD-ase L is shown **(SEQ ID NO: 35)**. Sites at which putative DNA sequences are spliced out are marked by an arrow with the resulting (in-frame) amino acid deletions highlighted in bold italicised letters **(SEQ ID NOS 37 & 38 respectively in order of appearance)**. Sites of putative intronic DNA insertion are marked by triangles, with the intronic DNA sequence shown above **(SEQ ID NOS 41, 43 & 45 respectively in order of appearance)**. Resulting changes to the amino acid sequence are highlighted in bold italicised letters **(SEQ ID NOS 42, 39, 44, 40 & 46 respectively in order of appearance)**. Numbering of amino acid residues is to the right.

Please delete the paragraph on page 12, lines 11-16 and replace it with the following paragraph:

Figure 4: is a nucleotide **(SEQ ID NO: 47)** and amino acid sequence **(SEQ ID NO: 48)** of human NAALAD-ase II. The nucleotide and predicted one letter code amino acid sequence are shown. The putative membrane spanning domain, deduced from hydrophilicity plots, is marked by a line. Potential N-glycosylation sites are shaded.

Please delete the paragraph on page 12, lines 18-23 and replace it with the following paragraph:

Figure 5: is a nucleotide sequence **(SEQ ID NO: 49)** and amino acid sequence **(SEQ ID NO: 50)** of human NAALAD-ase IV. The nucleotide and predicted one letter code amino acid sequence are shown. The putative membrane spanning domain, deduced from hydrophilicity plots, is marked by a line. Potential N-glycosylation sites are shaded.

Please delete the paragraph on page 12, lines 25-37 and replace it with the following paragraph:

Figure 6: is an alignment of the predicted protein sequences for human NAALAD-ases I **(SEQ ID NO: 51)**, L **(SEQ ID NO: 35)**, II **(SEQ ID NO: 48)** & IV **(SEQ ID NO: 50)**. The amino acid sequences were aligned using the ClustalW alignment program. Amino acid residues identical to all four proteins are shaded in black. Amino acid residues identical to three of the four proteins are shaded in grey. Amino acid residues are numbered to the right. A putative Zn^{2+} peptidase domain is highlighted between arrows and was identified by comparison to yeast and bacterial aminopeptidases. Putative residues involved in the catalytic site of the α/β hydrolase fold family of proteins are marked by three arrows (nucleophile-acid-base).

Please delete the paragraph on page 13, lines 8-28 and replace it with the following paragraph:

Figure 8: is an alignment of the NAALAD-ase peptidase domains with related peptidases. Amino acid sequences were aligned using the standard settings of CLUSTALW alignment program. Similar amino acid residues conserved in proteins are shaded in black. Similar

amino acid residues conserved in 80% of the proteins are shaded in dark grey. Similar amino acid residues conserved in 60-79% of the proteins are shaded in light grey. Amino acid residues are numbered to the right. Putative residues involved in zinc binding are marked by asterisks. The base residue thought to be important in catalysis is marked by an arrow. Sequence names other than NAALAD-ases **(SEQ ID NOS 56-59 respectively in order of appearance)** correspond to sequence accession numbers in Swiss-Prot and SPTREMBL; Ape 3 yeast **(SEQ ID NO: 52)**, Saccharomyces cervisiae aminopeptidase Y; P96152 **(SEQ ID NO: 53)**, Vibrio cholerae aminopeptidase ;. Ampx vibpr **(SEQ ID NO: 54)**, Aeromonoas proteolyitca aminopeptidase, Application strgr **(SEQ ID NO: 55)**, Streptomyces griseus aminopeptidase. Putative residues involved in zinc binding are marked by asterisks. General base residue thought to be important in catalysis is marked by an arrow.

Please delete the paragraph on page 18, line 36 to page 19, line 21 and replace it with the following paragraph:

Cloning of NAALAD-ase I by PCR. Sequence data from human NAALAD-ase I (Accession no. M99487) was used to design primers to amplify the complete coding sequence of NAALAD-ase I by PCR. Primers used were NAALD1S2 (BamHI) = 5'-CCC GGA TCC GAG ATG TGG ATT CTC CTT CAC GAA AC -3' **(SEQ ID NO: 1)** and NAALDIAS2 (XhoI) = 5'- CCC CTC GAG TTA GGC TAC TTC ACT CAA AGT CTC TGC -3' **(SEQ ID NO: 2)** (restriction sites to be introduced are underlined). PCR amplification was performed using Human Marathon-Ready$_{TM}$ cDNA from prostate in a total reaction volume of 50 µL, containing 1X Expand LongTemplate^{TM} PCR buffer 2, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers NAALD1S1 (BamHI) and NAALD1AS1 (XhoI), 1 µL of Marathon-Ready$_{TM}$ cDNA and 2.5 U of Expand Long Template PCR mix. Samples were pre-heated at 94°C for 5 min before addition of enzyme. Cycling was for 45 s at 94°C, 1 min at 55°C and 1 min 48 s at 68°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were analysed on a 1% agarose gel (wt/vol) in 1X TAE buffer (40 mM Tris-acetate, 1 mM EDTA (sodium salt), pH 8.3) and the most prominent DNA band was excised from the gel and purified with the Qiaquick gel extraction kit (Qiagen

GmbH,Dusseldorf, Germany).

Please delete the paragraph on page 19, line 28 to page 20, line 16 and replace it with the following paragraph:

Ligations were incubated overnight at 14°C. 2 µl of the ligation reaction was transformed into TOP10F' competent cells using heat-shock transformation and plated on 2x YT/ampicillin plates supplemented with IPTG and X-gal for blue-white screening. Colony screening was performed on 10 white colonies, from which plasmid DNA was prepared using the Qiagen plasmid mini DNA purification kit, and then digested with BAMHI and XHoI. Four clones containing an insert of the appropriate size were sequenced fully. All the clones had at least one mis-sense PCR error. Clone 10.0 with a single PCR error at position 1183 was used as the template for a site directed mutagenesis (SDM) reaction using the QuickChangeTM SDM Kit. Reactions were carried out according to the manufacturer's instructions. Primers designed for the amplification reactions were NAALD1-SDM-S1 = 5'-CCC TCA GAG TGG AGC AGC TGT TGT TCA TGA AAT TGT GAG G -3' **(SEQ ID NO: 3)** and NAALD1-SDM- AS1 =5'- CCT CAC AAT TTC ATG AAC AAC AGC TGC TCC ACT CTG AGG G -3' **(SEQ ID NO: 4)**. Three white clones from the SDM transformations were screened. Plasmid DNA was prepared using the Qiagen plasmid mini DNA purification kit, digested with BamHI and XhoI and sequenced around the mutation site. A single clone (cl. 2.0) was sequenced fully on both strands to confirm the complete correct NAALAD-ase I sequence.

Please delete the paragraph on page 20, line 18 to page 21, line 31 and replace it with the following paragraph:

Cloning of NAALAD-ase L by PCR and 5' rapid amplification of cDNA ends (RACE) PCR. Sequence data from partial human NAALAD-ase L (GenBank Accession no. AF10141) was used to design primers to amplify the 3' end of NAALAD-ase L by PCR. Primers used were NAALD2S1 = 5'- GTT CTT CAA CAA GCT GCA GGA GCG -3' **(SEQ ID NO: 5)** and NAALD2AS1(XhoI) = 5'- CCC CTC GAG CCG GAG TAA AGG GAG GGC TGA AG -3' **(SEQ ID NO: 6)**. HumanMarathon-Ready$_{TM}$ cDNA from brain, fetal brain, prostate, small intestine, colon were used in the amplification reactions. First round PCR amplification

was performed in a total reaction volume of 50 µl containing 1X Expand High Fidelity$_{TM}$ PCR buffer, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers NAALD2S1 and NAALD2AS1, 1 µl of Marathon-ReadyTM cDNA and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for 5 min before addition of the enzyme. Cycling was for 45 s at 94°C, 1 min at 58°C and 1 min at 72°C for 30 cycles, with a final step of 7 min at 72° C. PCR products were analysed on a 1% agarose gel in 1X TAE buffer. Second round PCR amplification was performed with nested primers NAALD2S2= 5'- GGC GAC CTG AGC ATC TAC GAC AAC -3' **(SEQ ID NO: 7)** and NAALD2AS2 (XhoI) = 5'-CCC CTC GAG TCC CCT CAG AGG TCA GCC ACA G -3' **(SEQ ID NO: 8)**. 1µl of the first round amplification reaction in a total volume of 50 µl containing 1X Expand High FidelityTM PCR buffer, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers NAALD2S2 and NAALD2AS2 and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for 5 min before addition of the enzyme. Cycling was for 45 s at 94°C, 1 min at 57°C and 1 min at 72°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were analysed on a 1% agarose gel in 1X TAE buffer and the most prominent DNA bands were excised from the gel and purified with the Qiaquick gel extraction kit. The resulting fragments 600-800 bp were cloned into CR2.1 as described previously. Ligations were incubated overnight at 14°C and transformed into TOP10F' competent cells and plated on 2x YT/ampicillin plates supplemented with IPTG and X-gal for blue-white screening. Colony screening was performed on five white colonies from each transformation. Plasmid DNA was prepared from these colonies using the Qiagen plasmid mini DNA purification kit and then digested with EcoRI. Plasmids containing inserts of the appropriate size were end sequenced using vector primers and then the full sequencing on both strands of putative NAALAD-ase L clones was performed. Clone 6.9 derived from small intestine extended to the translation termination codon.

Please delete the paragraph on page 21, line 32 to page 22, line 35 and replace it with the following paragraph:

To obtain unknown 5' coding sequence for human NAALADase L, two anti-sense primers were designed for 5' rapid amplification of cDNA ends (5'RACE). The primers were NAALD2AS3= 5'- GCC AGC ACC CAG AGA ACC CAA G -3' **(SEQ ID NO: 9)** and NAALD2AS4= 5'-GCT GCG GTT GAA GTA CCG GAT C -3' **(SEQ ID NO: 10)**.

HumanMarathon-Ready cDNA from brain, fetal brain, prostate, small intestine and colon were used for the 5' RACE according to the manufacturer's instructions. TheMarathon-Ready cDNA was prepared using oligo-dT priming and a Marathon cDNA adaptor (including two different adaptor-primer annealing sites) ligated to the 5' end of thecDNA. AdaptorprimerAP1 (5'- CCA TCC TAA TAC GAC TCA CTA TAG GGC3') **(SEQ ID NO: 11)** and nested adaptor-primer AP2 (5'- ACT CAC TAT AGG GCT CGA GCG GC -3') **(SEQ ID NO: 12)** were included in the kit.

Please delete the paragraph on page 22, lines 10-35 and replace it with the following paragraph:

First round PCR amplification was performed in a total reaction volume of 50 µl containing 1X Expand High Fidelity^{Tm} PCR buffer, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers AP1 and NAALD2AS3, 5 µl of Marathon-Ready^{TM} cDNA and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for 5 min before addition of the enzyme. Cycling was for 30 s at 94°C, 30 s at 58°C and 2 min at 72°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were analysed on a 1% agarose gel in 1X TAE buffer. Second round 5' RACE was performed using 1 µl of the first round amplification reaction in a total volume of 50 µl containing 1X Expand High Fidelity^{TM} PCR buffer, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers AP2 and NAALD2AS4 and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for min before addition of the enzyme. Cycling was for s at 94°C, 30 s at 57°C and 1 min at 72°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were excised from the gel and cloned into the vector pCR2.1 as described earlier. Colony screening by PCR was performed on 60 white colonies in 45 41 PCR mix containing 1X PCR buffer with M9Cl2, 0.2 mM dNTP, 0.5 µM each of vector primer M13FOR (5'- TGT AAA ACG ACG GCC AGT - 3') **(SEQ ID NO: 13)** and M13REV (51 - CAG GAA ACA GCT ATG ACC -3') **(SEQ ID NO: 14)** and 0.35 U of super Taq DNA polymerase.

Please delete the paragraph on page 23, line 32 to page 24, line 9 and replace it with the following paragraph:

Colonies were picked the following day, plasmid DNA prepared and tested by restriction digest. A single clone (cl. 2.0) was fully sequenced on both strands and found to contain the complete 3' coding sequence and the additional sequence from the first 5' RACE reactions. To obtain additional 5' coding sequence for the human NAALAD-ase L, two new anti-sense primers were synthesized corresponding to sequences from Incyte clone number 4190746. Primers used were NAALD2ASS = 5'- CTG CAG CTT GTT GAA CTC TTC TGT G -3' **(SEQ ID NO: 15)** and NAALD2AS6 = 5' CAA ACA CGA TTG ATC TGC GAG GAC – 3' **(SEQ ID NO: 16)**. Human Marathon-Ready™ cDNA from brain, fetal brain, prostate,small intestine, colon and heart were used for the 5' rapid amplification of cDNA ends (5' RACE) according to the manufacturer's instructions.

Please delete the paragraph on page 25, lines 15-28 and replace it with the following paragraph:

To construct a full length NAALAD-ase L clone, two new primer sets were designed to introduce a unique restriction site (Mun I) into the DNA sequence of NAALAD-ase L without resulting in a change in amino acid sequence or frame shift in the open reading frame (ORF). The first primer set was NAALD2S3 (EcoRV) = 51- CGG ATA TCC GCA GGA TGC AGT GGA CGA AG -3' **(SEQ ID NO: 17)** and NAALD2AS8 (MunI) - 5'- CAA ACA CAA TTG ATC TGC GAG GAC GC -3' **(SEQ ID NO: 18)** and the second primer set was NAALD2S8 (MunI) '- GCG TCC TCG CAG ATC AAT TGT GTT TG -3' **(SEQ ID NO: 19)** and NAALD2AS1 (XhoI). PCR amplification was performed on 1 µL cl. 2.0 plasmid DNA with primers NAALD2S3 (EcoRV) and NAALD2AS8 (MunI) or on 1 µl cl. 2.2 plasmid DNA with primers NAALD2S8 (MunI) and NAALD2AS1 (XhoI).

Please delete the paragraph on page 26, line 11 to page 27, line 18 and replace it with the following paragraph:

Sequencing results from Incyte clone 3608639 suggested that this clone contained DNA sequence spanning the complete coding sequence, 2220 bp in size, of a putative NAALAD-ase like molecule (NAALAD-ase II) that had similar sequence to NAALAD-ase I and L. To confirm that there was no possible initiation codon upstream of the initiation codon already determined 5' RACE PCR was performed. Two anti-sense primers were

designed for 5' RACE based on the sequence derived from the clone 3608639, NAALD3ASI = 5'- CTT TGA TGA TAG CGC ACA GAA GTG G -3' **(SEQ ID NO: 20)** and NAALD3AS2 = 5' GGA AAG ATG CCA GCG CAG GAC 03' **(SEQ ID NO: 21)**. Human Marathon-Ready™ cDNA from brain, foetal brain, prostate, small intestine and colon were used for the 5' RACE according to the manufacturer's instructions. First round PCR amplification was performed in a total reaction volume of 50 µl containing 1X Expand High Fidelity™ PCR buffer, 0.2 mM of each dNTP, 02 µM each of oligonucleotide primers AP1 and NAALD3AS1, 5 µl of Marathon- Ready™ cDNA and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for 5 min before addition of the enzyme. Cycling was for 30 s at 94°C, 30 s at 58°C and 2 min at 72°C for 30 cycles, with a final step of 7 min at 72°C. Second round 5' PACE was performed using 1 µl of the first round amplification reaction in a total volume of 50 µl containing 1X Expand High Fidelity™ PCR buffer, 0.2 mM of each dNTP, 0.2 µM each of oligonucleotide primers AP2 and NAALD3AS2 and 2.5 U of Expand High Fidelity PCR mix. Samples were preheated at 94°C for 5 min before addition of the enzyme. Cycling was for 30 s at 94°C, 30 s at 57°C and 1 min at 72°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were analysed on a 1% agarose gel in 1X TAE buffer and the most prominent DNA bands were excised from the gel and purified with the Qiaquick gel extraction kit. The resulting fragments (250 - 600 bp) were cloned into the plasmid pCR2.1 as described previously. 32 white colonies were grown overnight in 3 ml LB medium supplemented with 100 µg/ml of ampicillin and plasmid DNA was prepared using the Qiagen plasmid mini DNA purification kit. No upstream initiation codon was identified from any of the 32 clones analysed.

Please delete the paragraph on page 27, line 21 to page 28, line 20 and replace it with the following paragraph:

Seqencing results from Incyte clone 2615389 revealed that this clone contained partial coding sequence and 3' UTR, of another putative NAALAD-ase like molecule (NAALAD-ase IV) that was related in sequence to NAALAD-ase I, L and II. The DNA sequence obtained was used in a BLAST search on the Incyte LifeSeqTm EST database. One contig (2519841) was assembled from 150 overlapping Incyte EST sequences that spanned 1881 bp and contained a coding region of 1419 bp. The sequence data from

human NAALAD-ase IV contig 2519841 was used to design primers to amplify the complete coding sequence of by PCR. Primers used were NAALD4SP2 = 51- CGT CAG AGC CGC CCT ATC AGA TTA TC -3' **(SEQ ID NO: 22)** and NAALD4AP4 '- GAG GAG TTT TCC AAA GTT GCA GAC CC -3' **(SEQ ID NO: 23)**. PCR amplification was performed using a human hippocampal cDNA in a total reaction volume of 50 µl, containing 1X Expand High Fiedlity™ PCR buffer, 0.2mM of each dNTP, 0.2 µM each of oligonucleotide primers NAALD4SP2 and NAALD4APA4, 1 µl of cDNA and 2.5 U of Expand High Fidelity™ PCR mix. Samples were pre-heated at 95°C for 5 min before addition of enzyme. Cycling was for 45 s at 94°C, 1 min at 58°C and 35 s at 72°C for 30 cycles, with a final step of 7 min at 72°C. PCR products were analysed on a 1% agarose gel in 1X TAE buffer and the most prominent DNA band was excised from the gel and purified with the Qiaquick gel extraction kit. The resulting 1544 bp DNA fragment was sub- cloned into the plasmid vector pCR2.1-TOPO using the TA TOPO cloning it according to the manufacturer's instructions. Approximately 10 ng of purified fragment was ligated to 10 ng of pCR2.1-TOPO plasmid DNA. Ligations were incubated for 5 min at 25°C. Transformations into TOPIOF1 competent cells and colony screening was performed as previously described. Three clones containing an insert of the correct size were sequenced fully and two clones were found to contain no PCR errors (cl. 28.0 and cl.1.0).

Please delete the paragraph on page 33, line 5 to page 34, line 5 and replace it with the following paragraph:

Oligonucleotide primers designed for the specific amplification of a PCR fragment for each NAALD-ase; NAALAD-ase I primers were NAALAD1S3 5' - GGG AAA CAA ACA AAT TCA GCG GC - 3' **(SEQ ID NO: 24)** and NAALD1AS3 51 GTC AAA GTC CTG GAG TCT CTC ACT GAA C - 3' **(SEQ ID NO: 25)** yielding a 341 bp product, NAALAD-ase L primers were NAALD2S7 51- GAC CGG AGC AAG ACT TCA GCC AG - 3' **(SEQ ID NO: 26)** and NAALD2AS7 5'- GTG TTG ATA TGC GTT GGC CCA AG - 3' **(SEQ ID NO: 27)** yielding a 330 bp product, NAALAD-ase II primers were NAALD3S4 51 CAC TAA GAA TAA GAA AAC AGA TAA GTA CAG C-3' **(SEQ ID NO: 28)** and NAALD3AS4 51-GAT CAA CTT GTA TAA GTC GTT TAT GAA AAT CTG - 3' **(SEQ ID NO: 29)** yielding a 353 bp product and NAALAD-ase IV primers were NAALD4SI S' - GCA GAA GAA CAA GGT GGA GTT GGT G- 3' **(SEQ ID NO: 30)** and NAALD4ASI 5' - GCT TTG GAT CCA TGA CAG TCA

TGG- 3' **(SEQ ID NO: 31)** yielding a 336 bp product. Each primer set for each NAALAD-ase was tested for its ability to specifically amplify that NAALAD-ase and not to cross react in amplification reactions with the other three forms. PCR amplifications for human GAPDH were performed on the same cDNA samples as positive controls using GAPDH specific primers 5'-TGA AGG TCG GAG TCA ACG GAT TTG GT-3' **(SEQ ID NO: 32)** (sense primer) and 5'- CAT GTG GGC CAT GAG GTC CAC CAC-3' **(SEQ ID NO: 33)** (anti-sense primer), yielding a 1000 bp fragment. These primers sets were used for PCR amplifications on human multiple tissue cDNA (MTCTM) panels normalised to the mRNA expression levels of six different housekeeping genes. Human cDNAs from 15 brain regions were also prepared from mRNA and normalised to the mRNA expression levels of three different housekeeping genes, GAPDH, clathrin and actin. Brain area mRNA was prepared starting from carefully dissected tissue samples, using the FastTrackR 2.0 kit (Invitrogen BV, Netherlands) according to the manufacture's instructions. 1µg of poly (A) + RNA was reverse transcribed using oligo (dT) 15 as a primer and 50 U of Exapnd™ Reverse Transcriptase (Boehringer Mannheim, Germany) according to the manufacturer's instructions.